Synthesis and Inhibitory Activities of Isochromophilone Analogues against gp120-CD4 Binding

Xue-Long Sun, Hiroaki Takayanagi*, Keichi Matsuzaki, Haruo Tanaka and Kimio Furuhata
School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108, Japan

Satoshi Omura
Research Center for Biological Function, The Kitasato Institute, 5-9-1 Shirokane, Minato-ku, Tokyo 108, Japan

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Several isochromophilone analogues were synthesized from sclerotiorin (1) by Wittig reactions and aldol condensation reaction. The structures of the products were elucidated from MS, elemental analysis, 1H NMR and 13C NMR spectra, and their inhibitory activities against gp120-CD4 binding were determined.

Sclerotiorin (1) occurs as a major metabolite in several species of monoverviciellate Penicillia. It was first isolated from a culture of Penicillium Van Beyma by Curtin and Reilly. Recently, in the screening program for new inhibitors against gp120-CD4 binding from microorganisms, isochromophilones Ia (2) and IIa (3), which have the same azaphilone skeleton and chlorine as 1, were found as novel non-peptide inhibitors from a culture broth of Penicillium sp. FO-2338 (Chart 1). Although 1 was also found to be produced abundantly by the strain, its inhibitory activity against gp120-CD4 binding was much less than that of 2 and 3. In order to understand the relationship between the structure and activity, we selected 1 as starting material to synthesize several derivatives, and determined their inhibitory activities against gp120-CD4 binding.

Results

The comparison of the structures of sclerotiorin (1) with isochromophilone Ia (2) and isochromophilone IIa (3) suggested that the 7-O-group and carbon-8 may be the groups principally responsible for the inhibitory activities. In order to prove this, at first, we tried the Wittig reaction at 8-carbonyl group of 1 with carbethoxy-methylene-triphenylphosphorane. However, we didn't succeed by using 1 as starting material directly, so we performed the basic hydrolysis of the 7-O-acetyl group of 1 with sodium methoxide in refluxing methanol to obtain 4 in 89% yield. From 4 we obtained an olefinic ester 5 in 70% yield. The structure of 5 was elucidated from MS and 1H NMR data. In 1H NMR, the characteristic signal of the new product is the new olefinic proton (6.59 ppm, s, 10-H) and O-ethyl group (4.15 ppm, m, CH2; 1.20 ppm, t, J=7.8 Hz, CH3). The sequcis conformation of the new olefin of 5 was determined by NOE experiment between H-1 and H-10 (2%). Treatment of 4 with the Wittig agent 1-triphenylphosphoranylidene-2-propanone gave 6 in 30% yield. 5 was acetylated by acetic anhydride in pyridine at room temperature to yield 7 in 91% yield. Compound 8 was synthesized from 5 under basic condition by intramolecular esterification in 85% yield. The structure was elucidated from MS, 1H NMR data, H-10 showed at 5.99 ppm (s), and NOE between H-1 and H-10 was approximately 5%.
To synthesize rubrorotiorin (9), we conducted an intermolecular esterification reaction between 4 and diketene, and then an intramolecular aldol condensation reaction of the above product (Scheme 2). From this reaction 10 was also obtained as by-product in 3% yield. The structure of these products were elucidated by MS, $^1$H NMR, $^{13}$C NMR data, HMBC, HMQC and NOE. For the above products 5, 6, 7, 8 and 9, we conducted selective reduction of C-8, C-10 olefin as $\alpha,\beta$-unsaturated carbonyl compounds with Birch reduction or sodium hydrogen telluride reduction reagent, but didn't succeed. It may be due to several olefin bonds and lactone structure in these compounds are unstable to these reduction conditions.

The inhibitory activities against gp120-CD4 binding of these products were determined. Table 1 shows that compounds 5, 8 and 9 inhibited gp120-CD4 binding with IC$_{50}$ of 2.6, 8.1, and 13 $\mu$g/ml, respectively. These values rank with those of 2 and 3 (IC$_{50}$: 2.75 and 1.51 $\mu$g/ml, respectively). Compound 4 exhibited weaker inhibition (IC$_{50}$: 39 $\mu$g/ml), but compound 10 exhibited no inhibition at more than 100 $\mu$g/ml.

In conclusion, we have selected 1 as starting material to synthesize several isochromophilone analogues, and determined their inhibitory activities against gp120-CD4 binding. These results suggested that 7-hydroxyl group may be effective for the inhibition, but 8-carbonyl group may be not. Further studies will be reported later.

### Experimental

#### General Procedures

Melting points were measured on a Yamato melting point apparatus and not corrected. Fast atom bombardment mass spectra (FAB-MS) were taken on JEOL JMS-DX 300. IR spectra were obtained on a Perkin-Elmer 983G Infrared spectrometer. The $^1$H NMR spectra were determined with a Varian VXR-300 and XL-400 spectrometers, in the solvent state, with tetramethyl-
silica (TMS) as an internal reference. Thin layer chromatography (TLC) was performed on kiesel gel 60F254 (Merck) plates, and spots were detected by ultraviolet (UV) and by spraying with 5% sulfuric acid solution. Column chromatography was conducted on silica gel 60 (70 ~230 mesh) (Merck).

7-Hydroxy-5-chloro-3-(3,5-dimethyl-1,3-heptadienyl)-7-methyl-6H-2-benzopyran-6,8(7H)-dione (4): 1 (500 mg, 1.30 mmol) was dissolved in anhydr. ether (30 ml), and Ph3P=CHCO2Et (411 mg, 1.28 mmol) was added. The mixture was processed as described for 4 to yield 5 (340 mg, 89%).

4: Yellow powder, mp: 80 ~82°C, FAB-MS m/z: 349 (M+1)+ (m-NBA as matrix), Anal. Caled for C19H21O4Cl: C 65.61, H 6.03, Cl 10.06. Found: C 65.29, H 6.01, Cl 9.72.

1H NMR (300 MHz, CDCl3) δ 6.86 (3H, t, J = 7.8 Hz, 7’-CH3), 1.05 (3H, d, J = 7.2 Hz, 9’-CH3), 1.30 (1H, m, 2-H), 1.44 (1H, m, 6-H), 1.58 (3H, s, 9-CH3), 1.84 (3H, s, 8’-CH3), 2.48 (1H, m, 5’-H), 3.80 (1H, brs, 7-CH3), 5.73 (1H, d, J = 10.2 Hz, 4’-H), 6.08 (1H, d, J = 15.6 Hz, 1’-H), 6.62 (1H, s, 4-H), 7.09 (1H, d, J = 15.6 Hz, 2’-H), 7.93 (1H, s, 1-H).

5-Chloro-3-(3,5-dimethyl-1,3-heptadienyl)-7-hydroxy-7-methyl-8-(ethoxycarbonylmethylidene)-6H-2-benzopyran-6-one (5): 4 (400 mg, 1.15 mmol) was dissolved in anhydr. ether (30 ml), and Ph3P=CHCOOC2H5 (419 mg, 1.28 mmol) was added. The solution was processed as described for 4 to yield 5 (340 mg, 70%).

5: Yellow powder, mp: 155 ~157°C, FAB-MS m/z: 419 (M+1)+ (m-NBA as matrix), Anal. Caled for C23H27O4Cl: C 66.03, H 6.46, Cl 8.37. Found: C 65.71, H 6.69, Cl 8.27.

1H NMR (300 MHz, CDCl3) δ 6.85 (3H, t, J = 7.8 Hz, 7’-CH3), 1.05 (3H, d, J = 7.2 Hz, 9’-CH3), 1.20 (3H, t, J = 7.8 Hz, 10-COOC2H5), 1.28 (2H, m, 6-H), 1.44 (3H, s, 9-CH3), 1.83 (3H, s, 8’-CH3), 2.47 (1H, m, 5’-H), 4.15 (2H, m, 10-COOC2H5), 4.27 (1H, brs, 7-CH3), 5.66 (1H, d, J = 10.2 Hz, 4’-H), 6.08 (1H, d, J = 15.6 Hz, 1’-H), 6.59 (1H, s, 10-H), 7.08 (1H, d, J = 15.6 Hz, 2’-H), 8.07 (1H, s, 1-H).

5-Chloro-3-(3,5-dimethyl-1,3-heptadienyl)-7-hydroxy-7-methyl-8-(2-oxo-propyliden)-6H-2-benzopyran-6,8(7H)-dione (8): 5 (330 mg, 1.24 mmol) was dissolved in anhydr. ether (30 ml), and NaOMe (158 mg, 2.50 mmol, 2 eq) was added. The mixture was stirred at room temperature for 1 hour, until no more starting material was found by TLC (n-hexane: acetone 3:1). The solution was poured into 5 n HCl aq. (20 ml), extracted with EtOAc (30 ml x 3), the extract was washed with saturated NaHCO3 aq. and brine, dried with anhydrous Na2SO4, concentrated to dryness, and purified by silica gel column chromatography (n-hexane: acetone 3:1) to yield 8 (250 mg, 85%).

8: Yellow amorphous, FAB-MS m/z: 373 (M+1)+ (m-NBA as matrix), Anal. Caled for C21H23O4Cl: C 67.74, H 5.65, Cl 9.41. Found: C 67.82, H 5.57, Cl 9.70.

1H NMR (300 MHz, CDCl3) δ 0.87 (3H, d, J = 7.8 Hz, 7’-CH3), 1.02 (3H, d, J = 7.2 Hz, 9’-CH3), 1.34 (1H, m, 6-H), 1.44 (1H, m, 6’-H), 1.67 (3H, s, 9-CH3), 1.85 (3H, s, 8’-CH3), 2.48 (1H, m, 5’-H), 5.71 (1H, d, J = 10 Hz, 4’-H), 5.99 (1H, s, 10-H), 6.09 (1H, d, J = 15.6 Hz, 1’-H), 6.58 (1H, s, 4-H), 7.08 (1H, d, J = 15.6 Hz, 2’-H), 7.63 (1H, s, 1-H).

9-Acetyl-5-chloro-3-(3,5-dimethyl-1,3-heptadienyl)-6a-methyl-6H-furo[2,3-f]-2-benzopyran-6,8(6aH)-dione (9): 4 (715 mg, 2.05 mmol) was dissolved in THF...
(50 ml), Diketene (345 mg, 4.1 mmol, 2 eq) and DMAP (20 mg, 0.16 mmol, 2 eq) were added. The solution was stirred at room temperature for 5 hours, until no more starting material was found by TLC (n-hexane:acetone 3:1). The reaction solution was evaporated to dryness in vacuo, and purified by silica gel column chromatography (n-hexane:acetone 4:1) to yield 9 (460 mg, 54%) and 2,7-diacetyl-6-chloro-5-(2-oxo-5,7-dimethyl-3,5-nonenyl)-9a-methyl-2//-furo[2,3-/z]-l -benzo-pyran-2,8-dione (10) (28 mg, 3%).

9: Yellow amorphous, FAB-MS m/z: 415 (M+1) .

10: Yellow amorphous, FAB-MS m/z: 455 (M+1) .

Inhibition Experiments

The inhibitory activities against gp120-CD4 binding of these products were determined by enzyme-linked immunosorvent assay (ELISA) using recombinant soluble CD4 and recombinant gp120 as described by Gilbert, M., et al.7) The reagents CD4 and gp120 and anti-CD4 used in the assay were generous gifts from Genentech Inc. (CA, U.S.A.).

References


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